Behavioural Responses of *Acroneuria lycorias* (Ins., Plecopt.) Larvae to Acute and Chronic Acid Exposure

R.E. McNicol and E. Scherer

Central and Arctic Region
Department of Fisheries and Oceans
Winnipeg, Manitoba R3T 2N6

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BEHAVIOURAL RESPONSES OF *Acroneuria lycorias* (INS., PLECOPT.) LARVAE TO ACUTE AND CHRONIC ACID EXPOSURE

by

R.E. McNicol and E. Scherer

Central and Arctic Region
Department of Fisheries and Oceans
Winnipeg Manitoba R3T 2N6

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ABSTRACT

McNicol, R.E., and E. Scherer. 1987. Behavioural responses of Acroneuria lycorias (Ins., Plecopt.) larvae to acute and chronic acid exposure. Can. Tech. Rep. Fish. Aquat. Sci. 1512: iv + 10 p.

The effects of acute and chronic acid exposure on the locomotor activity, microdistribution, and drift behaviour of Acroneuria lycorias larvae were studied in laboratory streams. When subjected to a reduction in pH from 8.1 to 2.5 over an 8-h period, larvae showed little behavioural response down to pH 4.2. As the pH fell to 3.0, "head-rubbing" activity appeared and increased in frequency. At pH 3.0 and below, larvae showed increased gill-ventilatory movements and locomotor activity. Most larvae died within 14 h of exposure to pH 2.5; however, they did not abandon their preferred refuges before death. Larvae exposed to 5 pH levels between 4.5 and 8.2 for 30-50 d displayed no significant changes in locomotor activity, drift behaviour or microdistribution when compared to control animals. Our results indicate that later instar larvae of this species are relatively acid tolerant.

Key words: acid; locomotor activity; drift; respiration; sublethal effects; insects.

RÉSUMÉ

McNicol, R.E., and E. Scherer. 1987. Behavioural responses of Acroneuria lycorias (Ins., Plecopt.) larvae to acute and chronic acid exposure. Can. Tech. Rep. Fish. Aquat. Sci. 1512: iv + 10 p.

On a étudié, dans des cours d'eau simulés en laboratoire, les effets de l'exposition chronique et aiguë à un milieu acide sur l'activité locomotrice, la microdistribution et le modèle de déplacement dans le cours d'eau de larves d'Acroneuria lycorias. Celles-ci, lorsque le pH de leur milieu a été réduit de 8,1 à 2,5 au cours d'une période de 8 heures, n'ont manifesté à peu près aucune réaction comportementale jusqu'à un pH de 4,2. Lorsque le pH a atteint 3,0, les larves ont commencé à se "frotter la tête" et ce phénomène n'a fait que s'amplifier. Lorsque le pH est passé en-dessous de 3,0, on constatait une augmentation des mouvements branchiaux et ventilatoires ainsi qu'une intensification de l'activité locomotrice. La plupart des larves sont mortes dans les 14 heures suivant l'exposition à un pH de 2,5, mais aucune n'a quitté pour autant sa retraite de prédilection avant de mourir. Chez les larves exposées à 5 niveaux de pH entre 4,5 et 8,2 pendant 30 à 50 jours, aucun changement significatif n'a été relevé sur le plan de l'activité locomotrice, du modèle de déplacement dans le cours d'eau ou de la microdistribution par rapport aux sujets témoins. D'après nos résultats, les larves d'insectes de cette espèce, à des stades postérieurs, sont relativement tolérantes à l'acide.

Mots-clés: acide; activité locomotrice; déplacement biologique; respiration; effets sublétaux; insectes aquatiques.

INTRODUCTION

Deposition of acidic precipitation into poorly buffered streams results in a long-term, gradual decline in pH as the buffering capacity of the surrounding watersheds is exhausted. However, occasionally pulses of low pH water will inundate a stream (e.g. during rapid snowmelt) resulting in short-term, but frequently severe, declines in stream pH (Haines 1981).

Several studies report that abrupt depression in stream pH through experimental acid additions resulted in increased drift densities of some benthic macroinvertebrates (Herricks and Cairns 1974; Hall et al. 1980; Pratt and Hall 1981; Zischke et al. 1983). However, whether this drift reflects passive flushing of dead and moribund animals, or some behavioural response remains unclear. While some crustacean species will respond behaviourally to contact with low pH water (Costa 1967; Laughlan et al. 1978; Steiger and Raddum 1981; Williams and Moore 1982; France 1985), investigations into behavioural responses of insects have not been reported.

Our study was designed to investigate the responses of a perlid stonefly, Acroneuria lycorias (Newman), to acute and chronic acid exposure. Larvae of this species are common in streams and rivers impacted by acidic precipitation (Peckarsky 1979). It is also a suggested standard toxicity test species (APHA 1981), which in previous studies has proven to be very sensitive to some toxicants (Scherer and McNicol 1986).

MATERIALS AND METHODS

Mature stonefly larvae were collected from the Roseau River, Manitoba, and held in the laboratory in Heath Techna fish-egg incubator trays. These trays, stacked one upon the other, contained screened compartments in which larvae were confined. A 1-hp submersible pump sent temperature-controlled (range 14 ± 0.1°C) water (≈12 $L \cdot min^{-1}$) from a reservoir to the top tray; the water then flowed through the screened compartment within the tray and cascaded down through the remaining trays before returning to the reservoir. Larvae were fed lab-cultured Chironomus tentans larvae ad libitum three times a week.

Experiments were conducted in two side-by-side, open-ended, rectangular glass channels (120 cm L x 7.5 cm W x 12 cm H), each supplied with water recirculated from separate, temperature-controlled 315 L reservoirs (Fig. 1; further details in McNicol and Scherer 1985). A plexiglass plate (1 cm thickness), covered with a thin layer of clear, silica sand, was placed on the bottom of each channel to act as a substrate. Test animals were confined to a 75-cm long section of each channel by nylon screens (3-mm mesh size). A clear, glass plate (40 cm L x 7 cm W x 0.2 cm H) was placed on top of the substrate and supported 5 mm above the surface by four 1-mm diameter glass posts. The surface of each plate was painted with a thin, translucent layer of black epoxy paint. While providing a refuge attractive to the photophobic and thigmotactic A. lycorias, these plates were translucent enough to allow viewing of larval positions via a 45°-angled mirror placed beneath the channels.

Daytime lighting (700 lux; 0.01 watts·cm^{-2}) was provided by two 40-watt fluorescent and two 15-watt incandescent lamps, while at night the channels were illuminated by six 40-watt incandescent lamps fitted with infrared (IR) filters (λ690-1400 nm). An automatic dimming device controlled the 15-watt lamps, and produced simulated dawn/dusk periods of 20/30 min for the acute and 40/60 min for the chronic exposure experiments (photoperiod of 12.5 L : 11.5 D and 13 L : 11 D; for more details see McNicol and Scherer 1985).

We used carbon/UV-dechlorinated Winnipeg city water containing the following major ion concentrations ($\bar{x}$ ± SD): 603 ± 59 µM $Ca^{++} \cdot L^{-1}$, 290 ± 55 µM $Mg^{++} \cdot L^{-1}$, 145 ± 37 µM $Cl^{-} \cdot L^{-1}$, 88 ± 7 µM $Na^{+} \cdot L^{-1}$, and 36 ± 2 µM $K^{+} \cdot L^{-1}$. Hardness was equivalent to 893 ± 45 µM $CaCO_3 \cdot L^{-1}$, while dissolved inorganic carbon was 1483 ± 202 $\mu M \cdot L^{-1}$. This water is softer and has a lower dissolved ion concentration than Roseau River water (hardness equivalent to 2284 µM $CaCO_3 \cdot L^{-1}$, 4800 µM dissolved inorganic carbon$\cdot L^{-1}$, 1300 µM $Ca^{++} \cdot L^{-1}$, 934 µM $Mg^{++} \cdot L^{-1}$, 186 µM $Na^{+} \cdot L^{-1}$, 37 µM $K^{+} \cdot L^{-1}$). However, we have also found this species in Precambrian Shield streams with much lower hardness and dissolved ion concentrations. Larvae were always allowed at least one week acclimation to Winnipeg city water before being used.

ACUTE EXPOSURE EXPERIMENT

Responses of two groups of 16 acid-exposed larvae were compared to those of two control groups. Each group (mean larva total length ±SD: 20.6 ± 3.6 mm) was placed into a stream channel (water depth 4 cm; mid-water column current velocity ≈20 $cm \cdot s^{-1}$) and allowed to habituate for a 24-h period (no replacement water inflow). At 0900 h the following morning, and continuing over the next 24 h (day 1, pre-treatment period), the mirror images of the larvae were photographed with black and white IR film at 0.5-h intervals using a 35-mm SLR camera with motor drive, attached to an intervalometer assembly (for details see McNicol and Scherer 1985). Concurrently, stress responses not recordable on IR film (e.g. respiratory movements) were videotaped until 1900 h (lights out). For acid-treated groups, pH of the test reservoir was reduced from control levels (7.9-8.2) to pH 2.5 over an 8-h period commencing at 0900 h the next day. This was accomplished by the addition of a 1 N or 0.1 N H_2SO_4 solution over a 5-min period at half hour intervals (≈0.3 pH units decrease per half hour). Reservoirs were vigorously aerated during the pH-reduction to remove free CO_2 liberated into the water during acidification. Measurements of free [CO_2] (method of Stainton et al. 1977, as modified by Giles et al. 1984) and [O_2] were made immediately after acid addition. Larvae

remained exposed to pH 2.5 until 0900 h the next morning. Recording was continued over this second 24-h period (day 2, treatment period) as during day 1. Control groups were treated and recorded in the same fashion as above, except that the pH remained at ambient over day 1 and 2.

IR negatives were viewed with the aid of a photographic enlarger, and the percentage of larvae moving a distance of $\geq$0.5 body lengths between each photo (i.e. over a half-hour period) determined by manual measurement. This movement was used as a measure of locomotor activity. Some of these data were determined from videotapes due to a malfunction of the SLR camera apparatus. Also calculated from these tapes were percentages of larvae performing rhythmic up-and-down body movements (so-called "push-ups"; Wiley and Kohler 1984), rates of these movements, and percentage of larvae displaying "head-rubbing". "Push-ups" appear to be a mechanism by which some stonefly larvae enhance gill ventilation (Knight and Gaufin 1963). During "head-rubbing", a larva would bend its head to one side and proceed to rub the head and thorax with the middle and hind legs on that side. Due to lighting problems, downstream crawling and drifting were not recorded.

Percentage data were transformed ($y' = \arcsin \sqrt{y}$) to equalize variances (Snedecor and Cochran 1980). The difference between activity counts on days 1 and 2 were calculated at each half-hour interval for each group of larvae (each serving as its own control). These values were then summed to give hourly values which were used to test for differences in locomotor activity between acid-stressed and unstressed groups. Only locomotor activity data collected between 0900 and 2200 h for each of days 1 and 2 were statistically analysed due to missing data over one night during one replicate. The method of Rowell and Walters (1976) was used for the analysis of variance of these data, in accordance with the repeated measures nature of the data.

CHRONIC EXPOSURE EXPERIMENT

The effects of chronic exposure to four pH levels (4.5, 5.0, 5.5, 6.0) on the behaviour of A. lycorias were studied as follows. The pH treatments were tested in pairs (control 1 with pH 5.5; pH 5.0 with pH 6.0; control 2 with pH 4.5). Eighty larvae (mean total length ± SD: 20.1 ± 2.5 mm) were exposed to each of the first two treatment pH levels (Control 1, 5.5) in separate Heath Techna fish-egg incubator systems. Larvae for each treatment were housed in two trays each divided into two compartments (20 larvae per compartment). Water (Table 1) was recirculated from 175-L reservoirs through the trays via an external pump (≈12 $L \cdot min^{-1}$). Pre-acidified (pH <4.5) replacement water (≈1 $L \cdot min^{-1}$), stripped of free CO_2 (method described in Giles et al. 1984), was back-titrated with a 0.5 N NaOH solution using a radiometer TTT2 titrator plus a Radiometer SAS 1 switchbox. The pH in each set of trays was gradually reduced to the target pH over the next seven days. These pH's were then maintained for another three weeks before behavioural recording began. Trays were checked every two or three days for exuviae and dead larvae. After this period, 10 larvae were randomly selected from one compartment of each of the two test pH groups and placed into separate stream channels for one day of habituation followed by three days of observations. This was repeated every seven days until all four compartments of each treatment group had been sampled (four replicates). As testing of the first pair of treatment groups began, the same number of larvae were being exposed to the next pair (pH 5.0, 6.0) in the same manner as before. Subsequently, recordings of behaviour were also carried out as before. Exposure of larvae to the third pair of treatment pH's (control 2, pH 4.5) started two weeks later, followed as before by behavioural recording. The second group of control animals served to test for changes in behaviour, unrelated to treatment effects, over the duration of the experiment (5 months).

At 0900 h on the day after habituation, photographic recording began and continued over the next 72 h, as described for the acute experiment. The number of larvae drifting or crawling to the downstream screen were also recorded on videotape .

Locomotor activity was calculated as before. However, for these chronic exposure data, the x-y position coordinates of the head and abdomen of each larva were digitized from the IR negatives with a Hipad® digitizer, and the data stored on microcomputer diskettes. These data were fed through an SAS program (McNicol and Scherer 1985) to calculate the number of position changes at half hour intervals, which were then summed to give hourly values. A mean hourly value was then calculated from the three days of recordings. Raw mean counts were transformed ($y' = \sqrt{y + 0.5}$) to normalize the data and equalize variances (Snedecor and Cochran 1980). Statistical analyses were as described for the acute experiment, except that replicates were treated as blocks to account for possible effects due to differences in pre-exposure times among replicates. Since drifting and downstream crawling rarely occurred during daylight hours and only sporadically at night, total nighttime counts of these activities only were analysed in a randomized complete block design ANOVA (Snedecor and Cochran 1980).

RESULTS

ACUTE EXPOSURE EXPERIMENT

During the initial 2-h period of acid addition, free [CO_2] increased rapidly, reaching as high as 550 $\mu M \cdot L^{-1}$ (Fig. 2). Levels dropped quickly with vigorous aeration, until by 5 to 5.5 h later, concentrations were close to ambient (≈20 $\mu M \cdot L^{-1}$).

Apart from some "head-rubbing" observed among a few larvae during the initial period of acidification, no behavioural responses were evident among acid-exposed larvae until approximately pH 4.2 was reached, at which point

"head-rubbing" became pronounced and increased in frequency with decreasing pH (Fig. 2). "Push-up" movements did not commence until approximately pH 3.0, but increased rapidly thereafter (Fig. 2). Mean rate of "push-ups" increased from 9 to 34 per minute during this period. Only on one occasion during the pre-exposure period of the acid-exposed groups did a larva display "head-rubbing" or "push-up" movements. Neither behaviour was observed among control larvae. Locomotor activity was significantly elevated among acid-exposed larvae ($p<0.05$), but levels did not differ from controls until the pH was below 4.0 (Fig. 2). Thereafter locomotor activity increased sharply. The decrease about 3 h after pH 2.5 had been reached, probably reflected the onset of mortalities.

Movement out from under the glass plates during daytime hours (0600-1900 h) was rare among all groups of larvae; acid-exposed larvae remained under the plates throughout this period even though under obvious stress (high frequency of "push-up" and "head-rubbing" activity). Twenty-four hours after the start of acidification, 87.5 and 100% of the larvae in the two acid-exposed groups were dead (no control mortalities), yet 60.0 and 68.7% of the larvae were still under the glass plates. Those latter percentages were similar to those of the control larvae (62.5 and 68.7%) indicating that larvae did not abandon their preferred microhabitat prior to death from lethal acid exposure.

CHRONIC EXPOSURE EXPERIMENT

Among the first four treatment groups tested (pH 5.0, 5.5, 6.0. Control 1), mortality in individual tray compartments ranged from 0 to 25%, with no pH-related trend (ANOVA; $p>0.05$). Mortality rates were significantly higher among control 2 and pH 4.5 treatment groups, ranging from 25 to 50%. However, the rates did not differ between the two treatments. This suggests that these mortalities were unrelated to pH conditions. Reasons for these higher mortality rates are unknown, though they may be related to the advanced stage of development among these larvae at this time, as evidenced by the well-developed wing-pads on several larvae. In fact, 23% of the dead larvae among the latter two treatments died in mid-moult (two during emergence moult), compared to 11% among the earlier four treatment groups (none underwent emergence moult). Despite the higher mortality rates among some treatment groups, larvae in all treatment groups were apparently feeding normally, judging from direct observation and the amount of accumulated fecal material. During behavioural recordings, one larva died in one replicate of each of the control 2 and pH 4.5 treatments only. In both cases, the larva died 3 h prior to the end of recording.

A comparison of locomotor activity between the two control treatments (non-parametric rank-sum test, Hollander and Wolfe 1973) indicated that activity among control 2 larvae was higher than that of control 1 larvae ($p=0.058$). This suggests that some sort of seasonal or maturational changes did occur among larvae over the course of the experiment. Consequently, two separate ANOVA analyses were performed. One included treatments pH 5.0, 5.5, 6.0 plus control 1, and the other treatments pH 4.5 plus control 2. Neither analysis indicated any signficiant pH effect on locomotor activity, nor were any trends apparent (Fig. 3). Similarly, there was no significant pH effect on drift, downstream crawling or total downstream movement of larvae (sum of drift and downstream crawling), nor were any trends indicated (Fig. 4).

DISCUSSION

Subjecting a stream to a pH as low as 4.0 for several months (Hall et al. 1980; Pratt and Hall 1981) would likely result in some mortalities, particularly among mayfly species (see Bell and Nebeker 1969). Many of these dead and dying animals would then be flushed from the substrate, adding to the background invertebrate drift. However, several studies report that drift densities increase rapidly in response to experimental stream acidification, usually within 24 h of the start of acid addition (Herricks and Cairns 1976; Hall et al. 1980; Pratt and Hall 1981). Although this could suggest that a behavioural mechanism, such as change of microdistribution, locomotor activity or escape behaviour may be responsible, direct evidence is lacking. Unfortunately, common drift-collecting methods do not allow accurate estimation of the conditions (dead, moribund or unimpaired) of drifting organisms when captured.

Nevertheless, comparisons of drift rates into and out of acidified stream sections led Pratt and Hall (1981) to suggest that some stream benthos do "avoid" acidified water. Laboratory studies demonstrate that some crustaceans will show avoidance behaviour or become hyperactive starting at pH levels as high as 6.0 (Laughlin et al. 1978; Steiger and Raddum 1981; Williams and Moore 1982; France 1985). Either reaction in a stream environment could result in dislodgement of affected animals and an increase in drift densities. However, we observed no behavioural reaction to acute or chronic acid exposure by Acroneuria lycorias, except at very low, lethal pH levels (96 h-LC50 of 3.16-3.32 according to Bell and Nebeker (1969) and Butler et al. (1973); 30 d-LC50 of 3.85 according to Bell (1971)). Even though stoneflies in general are considered to be relatively acid tolerant (Bell and Nebeker 1969; Bell 1971; Hall et al. 1980), a lack of response at all but lethal pH levels is surprising. A few larvae displayed some response ("head-rubbing") during the initial period of acid addition, but this was very short-lived, and may have been a reaction to very high free CO_2 levels (≈25x ambient) generated from the reaction of hydrogen ions with bicarbonates in the water. Other aquatic invertebrates are known to respond to high free CO_2 levels (Scherer 1965; Costa 1967). However, it remains to be clearly demonstrated that any aquatic insect species responds behaviourally to sub-lethal exposure to elevated concentrations of hydrogen ions.

A further noteworthy observation in our study was that test animals did not abandon a habitat where they were exposed to acutely stressful and ultimately lethal pH conditions. As pH is depressed below a critical value, Ca, Na, and acid-base regulations in aquatic invertebrates are disrupted; further reductions hinder respiratory functions, with mortality following shortly thereafter (Havas 1981; Lechleitner et al. 1985). A. lycorias showed no visible signs of stress until lethal pH levels were reached, at which time respiratory problems were apparent. Despite being under obvious stress as pH was depressed even further, larvae made no attempt to seek a less stressful environment, i.e. abandon their preferred microhabitat (cf. Scherer 1965; Nagell 1977; Wiley and Kohler 1980), instead alleviating this respiratory stress by means of "push-ups". Yet in response to exposure to other chemical stressors, A. lycorias will leave a refuge. Under laboratory conditions identical to this study, larvae responded to very low concentrations of the insecticides methoxychlor and fenitrothion by abandoning their glass-plate refuges. In the case of methoxychlor this reaction occurred within 1-2 min after first contact (Scherer and McNicol 1986). Apparently, both sensitivity to different chemical stressors and the behavioural strategies for coping with stress can vary widely for this species.

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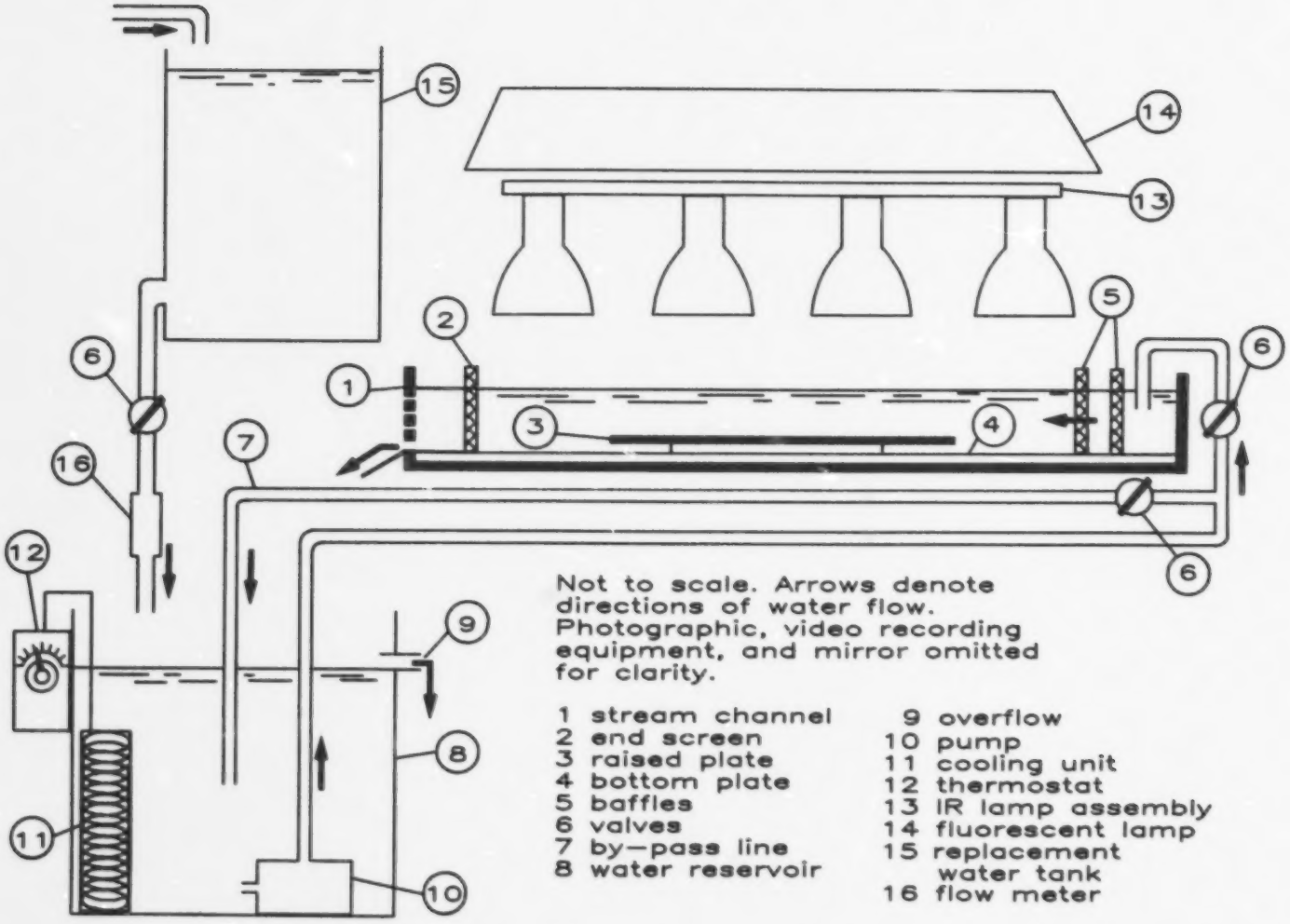
Table 1. Physical and chemical properties (ranges) of treatment water during exposure (exposure trays) and recording (stream channels) periods of chronic exposure experiment. Water temperature, pH and $[O_2]$ were measured daily; $[CO_2]$ and $[Na^+]$ were measured approximately weekly.

Treatment	Exposure period					Recording period				
	Temperature (°C)	pH	$[O_2]$ (ppm)	$[CO_2]$ ($\mu M \cdot L^{-1}$)	$[Na^+]$ ($\mu M \cdot L^{-1}$)	Temperature (°C)	pH	$[O_2]$ (ppm)	$[CO_2]$ ($\mu M \cdot L^{-1}$)	$[Na^+]$ ($\mu M \cdot L^{-1}$)
Control 1	14.1-15.4	7.86-8.01	9.4-10.2	30	84-90	13.8-14.0	7.89-8.19	9.9-10.2	30	80
Control 2	13.4-14.2	8.04-8.22	10.1-10.3	30-35	94-126	13.8-14.0	8.03-8.15	10.1-10.2	30-35	88-116
pH 6.0	14.3-16.6	5.85-6.08[1]	9.6-10.1	20-30	138-296	13.9-14.0	5.93-6.04	9.9-10.2	20	273
pH 5.5	14.4-15.8	5.40-5.60[2]	9.2-10.2	10-20	199-281	13.9-14.0	5.46-5.60	10.0-10.2	20	216
pH 5.0	14.1-16.4	4.90-5.09[1]	9.6-10.1	20	159-276	13.8-14.1	4.96-5.08	10.0-10.2	20	259
pH 4.5	13.9-14.0	4.43-4.70[3]	10.1-10.3	20-25	115-133	13.7-14.2	4.49-4.60	10.1-10.3	20	90-147

[1] pH rose to 7.5 and dropped to 3.9 on two separate occasions for a maximum of 17 h due to a malfunction of the pH control apparatus.

[2] pH rose to 6.0 and 7.9 for a maximum of 17 h, and to 6.6 and 7.2 for a maximum of 5 h, on four separate occasions due to a malfunction of the pH control apparatus.

[3] pH rose to 8.6 for a maximum of 5 h due to a malfunction of the pH control apparatus.



Not to scale. Arrows denote directions of water flow. Photographic, video recording equipment, and mirror omitted for clarity.

1 stream channel
2 end screen
3 raised plate
4 bottom plate
5 baffles
6 valves
7 by—pass line
8 water reservoir
9 overflow
10 pump
11 cooling unit
12 thermostat
13 IR lamp assembly
14 fluorescent lamp
15 replacement water tank
16 flow meter

Fig. 1. Schematic of stream channels, and water recirculating and lighting systems (from Scherer and McNicol 1986).

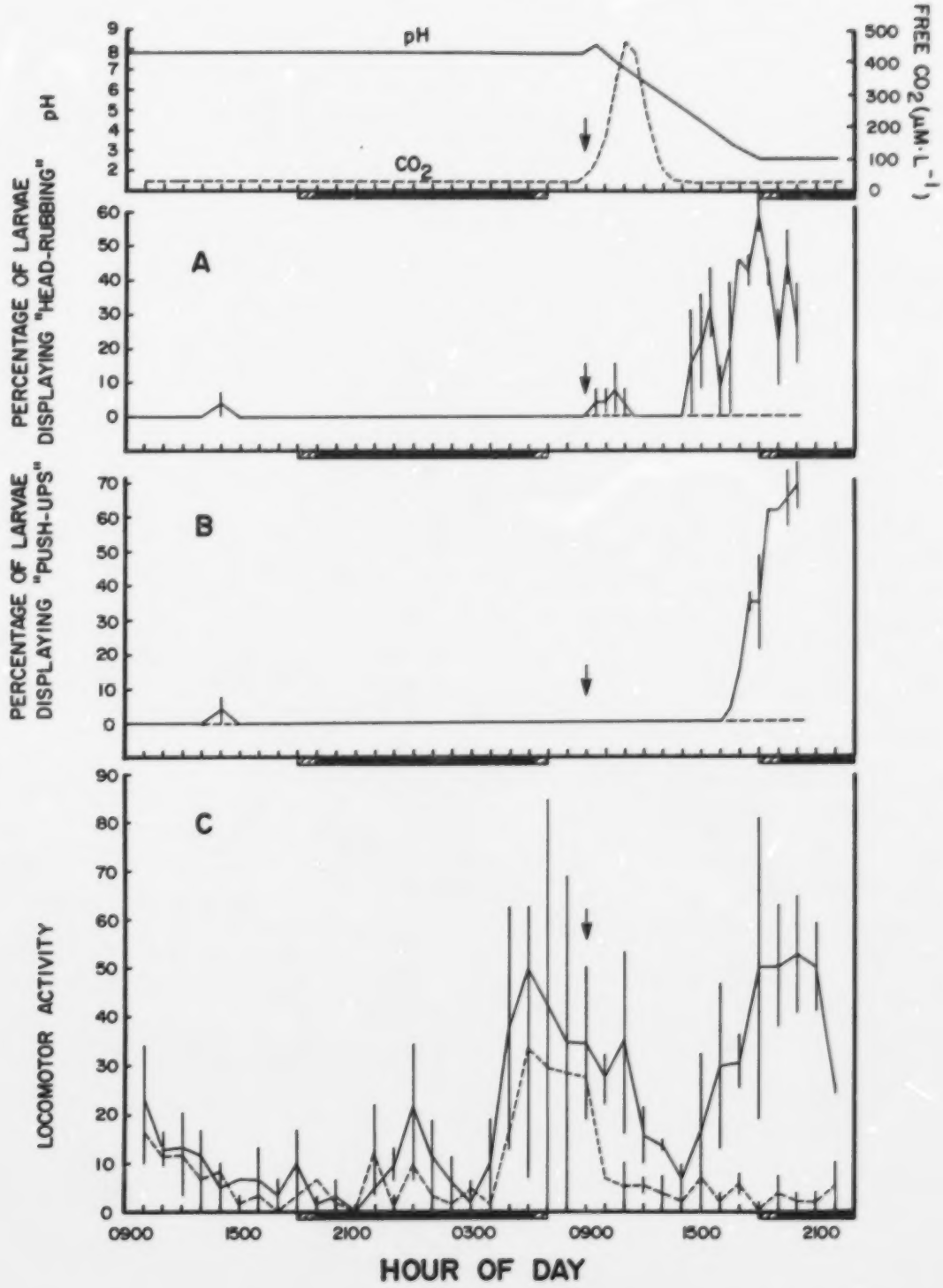


Fig. 2. Behavioural responses of Acroneuria lycorias larvae to rapid pH reduction. A) "Head-rubbing", B) "Push-ups", C) Locomotor activity (arrows indicate time at which pH reduction began; ---, control; ___, acid-exposed; raw mean ± SE).

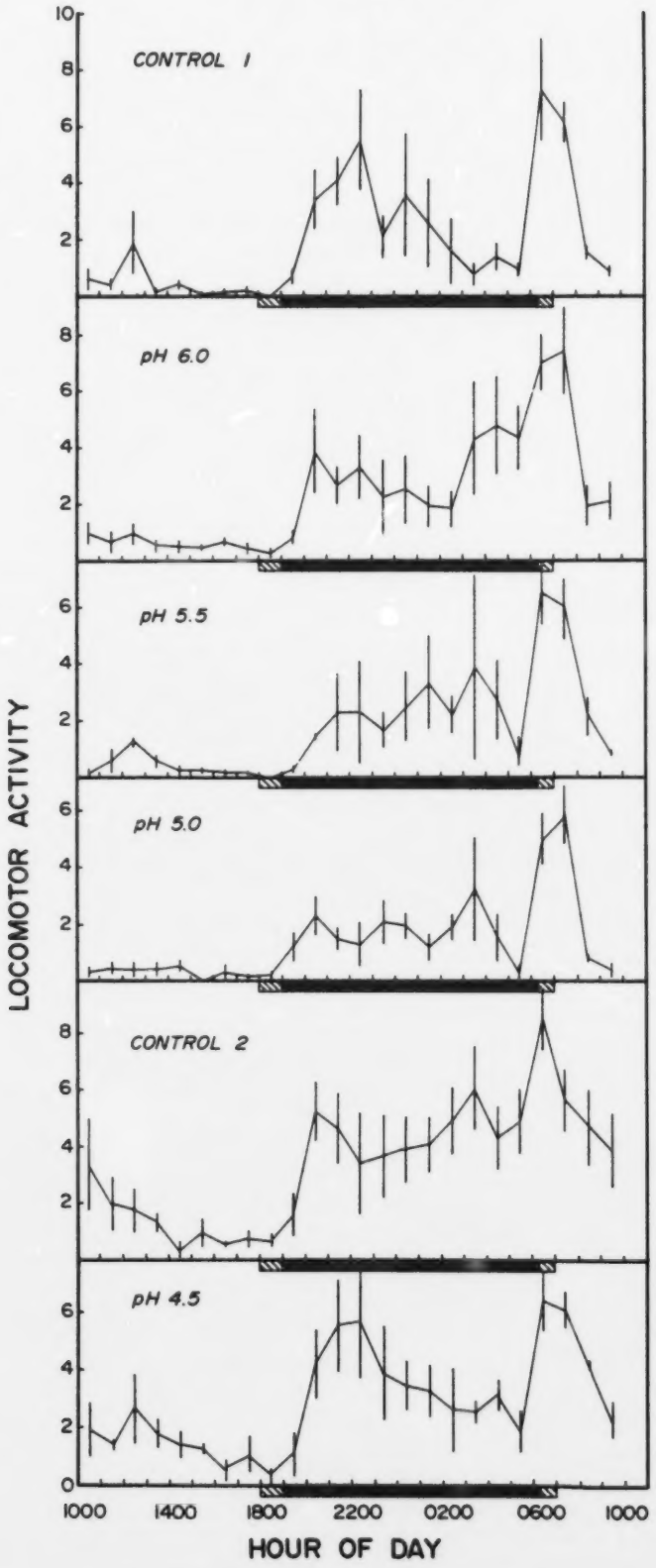


Fig. 3. Effect of chronic exposure to several pH levels on the locomotor activity of Acroneuria lycorias larvae (raw mean ± SE).

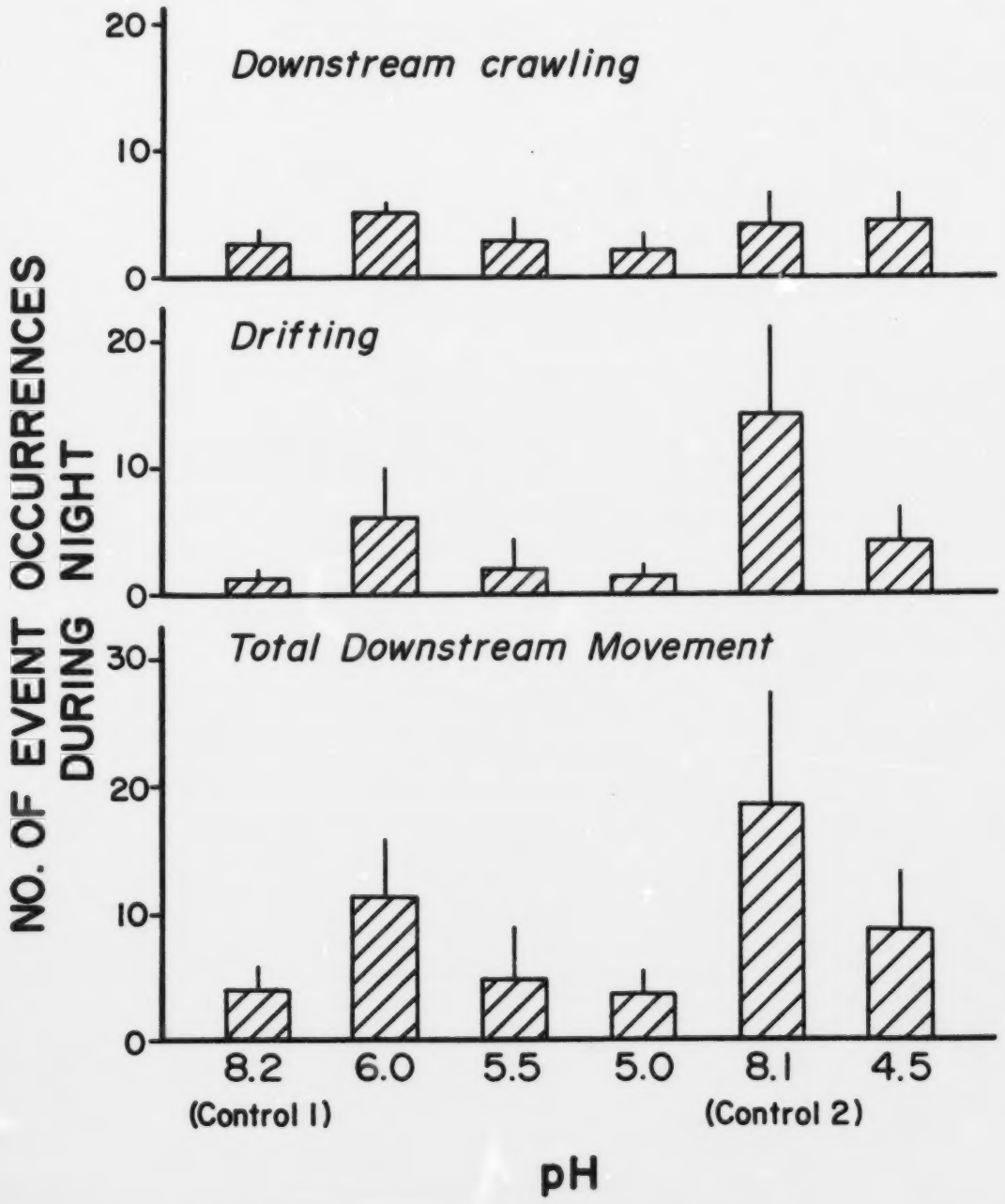


Fig. 4. Effect of chronic exposure to several pH levels on the downstream movement of Acroneuria lycorias larvae (raw mean ± SE).